

Microsite Factors and Spore Dispersal Limit Obligate Mycorrhizal Fern Distribution: Habitat Islands of *Botrychium pumicola* (Ophioglossaceae)

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ABSTRACT.—We investigated the influence of microsite and establishment factors on the distribution of *Botrychium pumicola* Coville ex Underw. (Ophioglossaceae), an obligate mycorrhizal fern restricted to specialized volcanic habitats, predominately pumice, in the Cascade Range of central and southern Oregon and northern California. The fragmented distribution of this threatened species and its absence from suitable habitat is of conservation concern. At eight subalpine and one alpine site, plots with and without *B. pumicola* were compared for biotic and abiotic variables. *Botrychium pumicola* populations occurred with diverse perennial plants. Nearest neighbor analysis showed a clumped dispersion of *B. pumicola* with associated plants. *Botrychium pumicola* spores were found in soils of seven plots with and two without *B. pumicola*. In plots with *B. pumicola*, soil potassium was higher while soil density and soil temperatures were lower than in plots without *B. pumicola*. Airborne spores, collected in spore traps, were dispersed up to 10 m from source plants. Microsite factors (soil nutrients, soil density, nearest neighbor dispersion) and dispersal limitations are likely key factors influencing the distribution of *Botrychium pumicola*. Narrow spore germination requirements combined with dispersal limitations may influence population expansion of obligate mycorrhizal ferns such as *B. pumicola*.

KEY WORDS.—*Botrychium*, endemism, mycorrhizal ferns, Ophioglossaceae, pumice, pumice moonwort, rare ferns, spore bank, spore dispersal

Causes of rarity in herbaceous plants are complex with multiple components (Stebbins, 1980; Kruckeberg and Rabinowitz, 1985; Fiedler, 1986). Proximate factors leading to rarity include intrinsic fitness (limited propagules, slow growth), requirement for mutualists (mycorrhizal symbionts, dispersal agents, pollinators), plant-soil interactions (inadequate soil components), plant-plant interactions (competition), meteorological factors (wind, precipitation, temperature), disturbance (changes in hydrology or nutrients, animal and human effects), and climate change (increased temperatures, changes in snowpack and moisture). The relative importance of these components has implications for conservation and rare species management. Factors such as intrinsic fitness and meteorological conditions are not subject to management; others such as disturbance, requirement for mutualists, and some of the effects of climate change may be ameliorated by management activities.

In ferns, the apparent ease of spore dispersal and the development of spore banks imply that microsite factors play a greater role than dispersal limitations

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in determining fern distribution (Tryon, 1970; Dyer and Lindsay, 1992; Kato, 1993). Habitat specificity is often implicated as the critical factor in fern distribution (Richard *et al.*, 2000; Guo *et al.*, 2003; Jones *et al.*, 2006). However, for some fern species, stochastic factors, including limited spore dispersal combined with habitat specificity influenced fern establishment (Peck *et al.*, 1990; Karst *et al.*, 2005; Wild and Gagnon, 2005). The distribution of obligate mycorrhizal ferns with narrow spore germination requirements (photoinhibition and arbuscular mycorrhizal inoculation) may be more influenced by microsite and establishment factors than other fern species. Conditions near established populations (available arbuscular mycorrhizal inoculum, similar soil nutrients and microclimate) are more likely to offer suitable habitat than more distant sites (Johnson-Groh and Lee, 2002; Karst *et al.*, 2005).

Botrychium pumicola Coville ex Underw. (Ophioglossaceae), pumice moonwort, is endemic to the Cascade Range of central to southern Oregon and northern California, and to the pumice plume areas of central Oregon (Farrar, 2012). A former Federal Category 1 Candidate for listing as Threatened and a former Federal Species of Concern, *B. pumicola* is listed as Threatened by the State of Oregon (USFWS, 1980; Lewis, 1996; Kagan *et al.*, 2010; ODA, 2012). It is on the U.S. Forest Service Region 6 Sensitive Species List, and is a U.S. Bureau of Land Management Special Status Plant and a California Special Status Plant (Hopkins *et al.*, 2001; ISSSSP, 2012; CDFG, 2013). First collected by Frederick V. Coville from Llao Rock in what would become Crater Lake National Park, *B. pumicola* grows in pumice fields, on pumice scree, and on volcanic rubble (Underwood, 1900; Wagner and Wagner, 1993; Hopkins *et al.*, 2001; Roe-Andersen, 2010). In spite of the presence of ripe sporangia with dispersed spores, this species is absent from adjacent pumice fields and other volcanic habitats that appear suitable (Hopkins *et al.*, 2001; Roe-Andersen, 2010).

Pumice soils derived from Mazama and Newberry plumes are characterized by low fertility, little soil profile development, and high porosity (Youngberg and Dyrness, 1964). In exposed pumice fields and open volcanic habitats, microtopographic features trap wind-blown materials and relief-driven moisture (rain and snow), creating microsites and favorable conditions for fern life cycle development (Körner, 1999; Titus *et al.*, 2007). Several flowering plant species including *Arnica viscosa* A. Gray (Asteraceae), *Boechera horizontalis* (Greene) Windham and Al-Shehbaz (formerly *Arabis suffrutescens* var. *horizontalis*, Brassicaceae), and *Astragalus peckii* Piper (Fabaceae) are endemic to pumice and volcanic substrates (Wolf and Denford, 1984; Windham and Al-Shehbaz, 2007; Martin and Meinke, 2012).

The goals of this study were to examine microsite factors and dispersal patterns that might affect the distribution of alpine and subalpine populations of *B. pumicola*. Specifically the objectives were to determine if environmental variables differed in areas inhabited by *B. pumicola* and in adjacent areas without *B. pumicola* and to evaluate dispersal capability. Our hypotheses are that: (1) adjacent plots with and without *B. pumicola* differ with regard to soil nutrients, soil texture, and site characteristics; (2) adjacent plots with and

without *B. pumicola* differ with regard to density of associated plant species, and *B. pumicola* spore bank; (3) *B. pumicola* dispersion is clumped; and (4) *B. pumicola* spore dispersal is predominantly local.

MATERIALS AND METHODS

Plant natural history.—Leaves of *Botrychium pumicola* emerge after spring snowmelt, with peak emergence mid-July to mid-August (Hopkins *et al.*, 2001; Roe-Andersen, 2010). Leaves reach heights of 1–6 cm above the soil surface, with 7–10 cm of the frond below ground. The stem with leaf primordia at the apex remains underground. The single leaf that matures each year is divided into a sterile trophophore and fertile sporophore. Reproduction occurs through spores, gemmae and intragametophytic selfing (Camacho, 1996; Camacho and Liston, 2001). Spores require a period of darkness for germination (Whittier, 1973; Johnson-Groh, 2002). Photoinhibition of spore germination ensures below-ground germination where the likelihood of sufficient moisture and proximity to mycorrhizal symbionts improve chances for colonization (Whittier, 2006).

Botrychium species form obligate symbioses with arbuscular mycorrhizal (AM) fungi, predominately *Glomus* spp. (Kovacs *et al.*, 2007; Winther and Friedman, 2007). Sporophytes of other *Botrychium* species remain underground for several years, relying on mycorrhizal partners to obtain fixed carbon from neighboring plants through an AM fungal network (Johnson-Groh, 1998; Winther and Friedman, 2007).

Study sites.—Populations were studied at eight subalpine sites and one alpine site: Broken Top (BR), Mt. Bachelor (BA), and the Dome (DO) in the Deschutes National Forest (DNF); Llao Rock with two populations, Llao East (LE) and Llao West (LW), Grotto Cove (GR), Skell Head (SK), Cloud Cap with two populations, Cloud Cap North (CN) and Cloud Cap South (CS), and Dutton Ridge (DU) in Crater Lake National Park (CLNP), Oregon; and Shastina Cone (SH) in the Shasta-Trinity National Forest (STNF), California (Fig. 1, Table 1; Roe-Andersen, 2010). Montane populations at lower elevations were not included. All sites receive heavy snow accumulation (up to 3 m) with average minimum temperatures below freezing for eight months of the year (Hopkins *et al.*, 2001; WRCDC, 2012). Average summer precipitation is low (3.1 cm total) and daily temperatures fluctuate widely (1.1–20.9°C). Plants are exposed to intense heat, cold, ultraviolet radiation, and strong winds.

Sites in CLNP were located in exposed, wind-swept pumice fields surrounding the caldera (Fig. 1B). Substrates consisted of volcanic “popcorn” pumice and mixed volcanic gravel from the Mt. Mazama pumice outfall c. 7700 years ago (Fig. 2A; Klimasauskas *et al.*, 2002). In the DNF, the subalpine site (DO) in Newberry National Volcanic Monument, is a volcanic cinder cone approximately 11,000 years old, covered with 0.5–1 m of Newberry “popcorn” pumice from the Big Obsidian Flow eruption c. 1300 years ago (Sherrod *et al.*, 1997). On the southeast flank of Broken Top Mountain, the BR site consisted of eroded andesite, dacite and rhyodacite from 100,000-year-old Broken Top

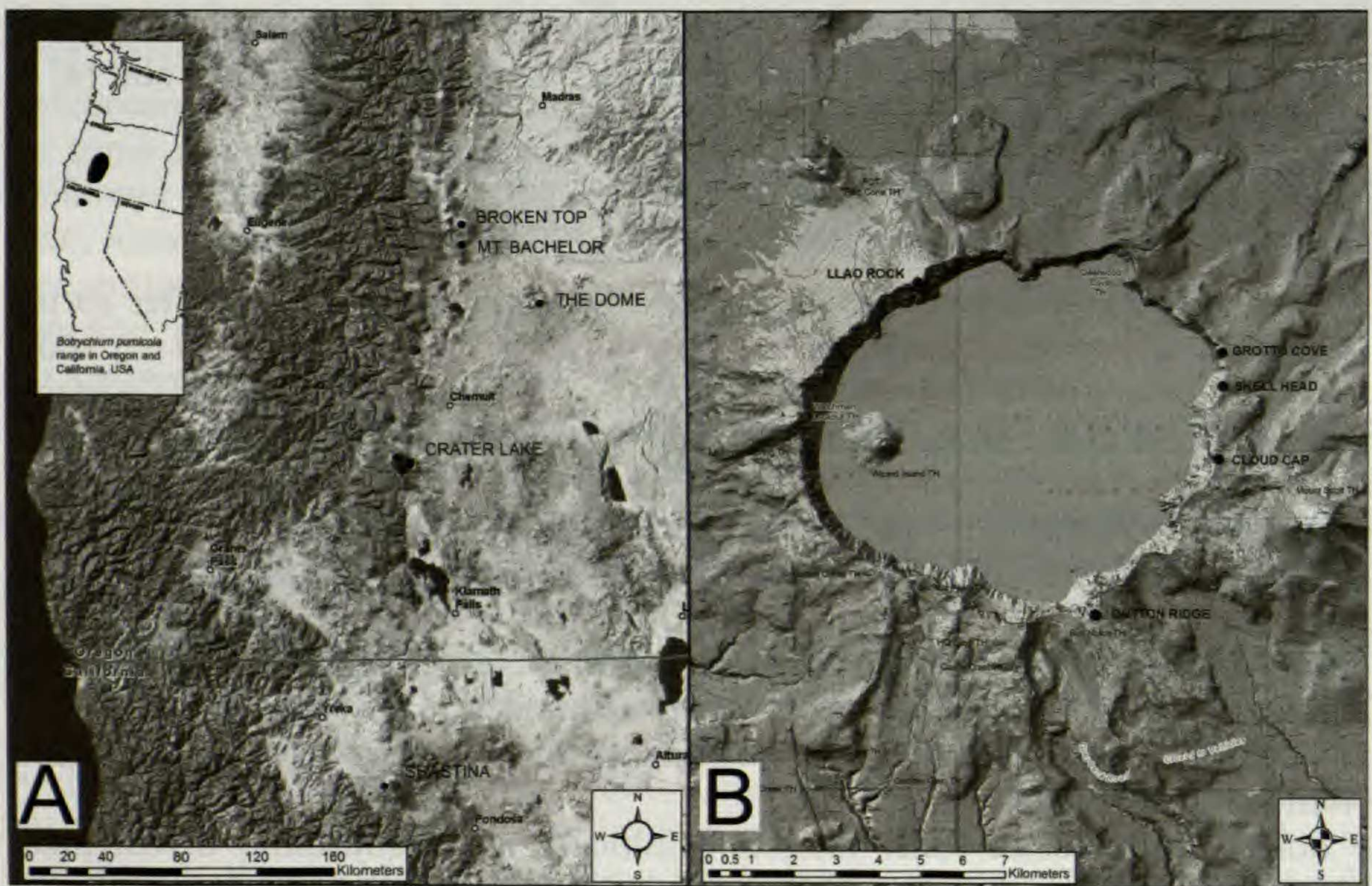


FIG. 1. *Botrychium pumicola* study sites. (A) The Cascade Range of central and southern Oregon and northern California. (B) Crater Lake National Park, Oregon.

eruptions, mixed with pumice and volcanic gravel from the South Sister eruption c. 2000 years ago (Scott *et al.*, 2003; Fig. 2B). The alpine site (BA) on the summit of Mt. Bachelor was in open, windswept volcanic rubble from Mt. Bachelor summit eruptions c. 12,000 years ago, and small amounts of pumice from the Mt. Mazama tephra plume c. 7700 years ago (Scott, 1990). The SH site was located on an exposed, windy, southwest flank near the upper end of Diller Canyon and consisted of andesitic boulder fields creating rock shelters for *B. pumicola* (Fig. 2C). This population was rediscovered 13 Jul 2009 (Eric

TABLE 1. *Botrychium pumicola* study sites arranged by elevation in the Cascade Range of central and southern Oregon and northern California. CLNP, Crater Lake National Park; DNF, Deschutes National Forest; STNF, Shasta-Trinity National Forest.

| Study Site | Location | Size (ha) | Elev (m) asl | Aspect | Slope | <i>B. pumicola</i> count |
|---------------|----------|-----------|--------------|----------------|--------|--------------------------|
| Grotto Cove | CLNP | 0.03 | 2100 | 12° | 3–10° | 13 |
| Skell Head | CLNP | 0.03 | 2180 | 250° | 7.5° | 37 |
| The Dome | DNF | 0.05 | 2190 | 18° | 12° | 75 |
| Broken Top | DNF | 0.10 | 2330 | 82°–140° | 15° | 636 |
| Cloud Cap | CLNP | 0.43 | 2400 | 185°–250° | 19–27° | 137 |
| Llao Rock | CLNP | 0.45 | 2440 | 110°, 250–290° | 0–19° | >1000 |
| Dutton Ridge | CLNP | 0.03 | 2460 | 190° | 9° | 1 |
| Shastina Cone | STNF | 0.03 | 2750 | 220° | 15–17° | 6 |
| Mt. Bachelor | DNF | 0.10 | 2760 | 129°–172° | 15° | 372 |

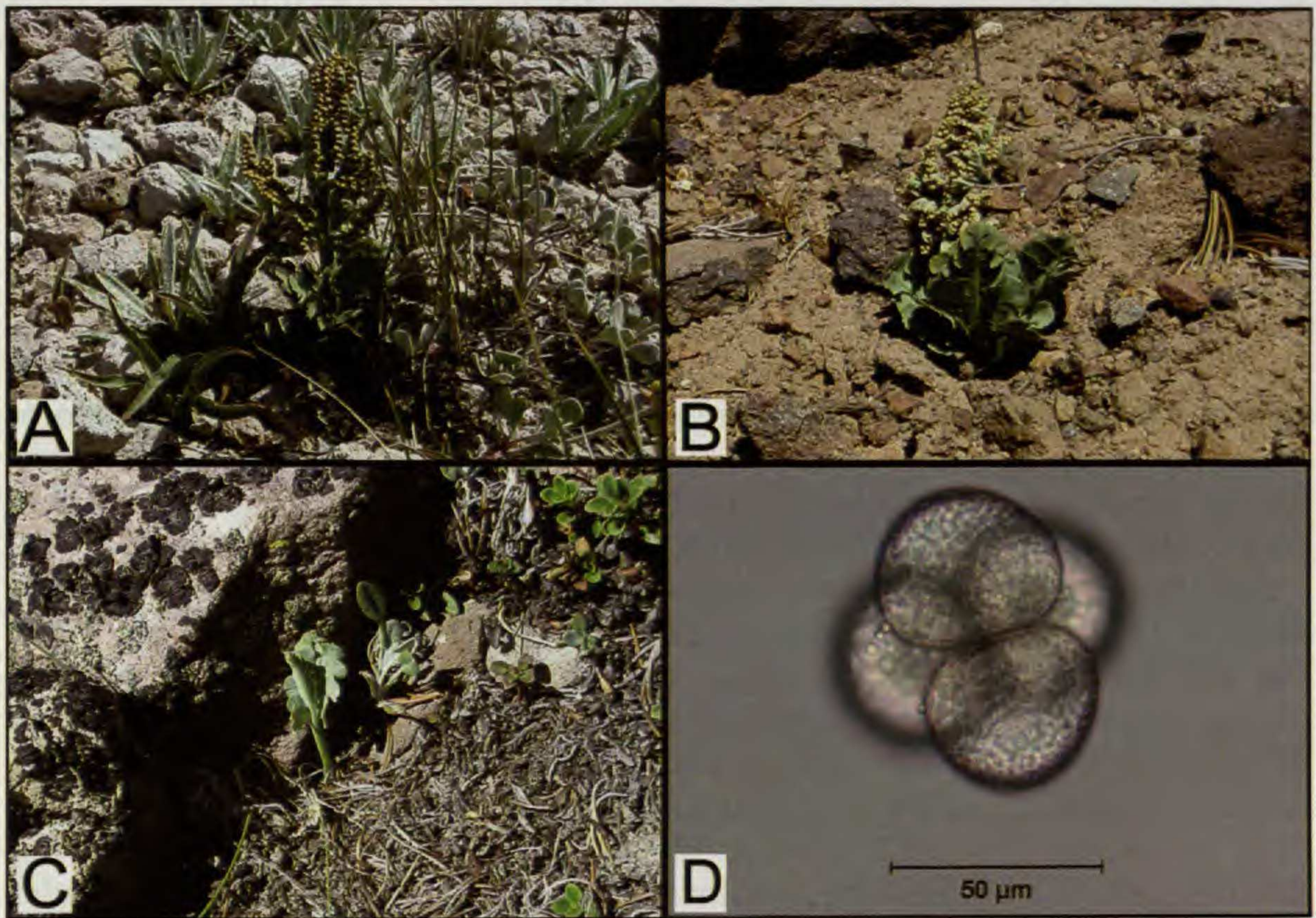


FIG. 2. *Botrychium pumicola* in the Cascade Range of central and southern Oregon and northern California. A–C, *B. pumicola* habitat. (A) Classic subalpine “popcorn” pumice, Liao Rock, Crater Lake National Park (CLNP). (B) Clay soil and few neighboring plants, Broken Top, Deschutes National Forest. (C) Andesitic rock shelter, Shastina Cone, Shasta-Trinity National Forest. (D) *B. pumicola* spore tetrad from soil sieving, Grotto Cove, CLNP.

White, U.S. Forest Service, pers. comm.). Shastina Cone formed 9700–9400 years ago as a subsidiary andesite cone on the west flank of Mt. Shasta (Christiansen, 1990).

Sampling design.—At each site, plots with *B. pumicola* were paired with adjacent plots without *B. pumicola*, but similar in slope, aspect, elevation, and vegetation. Because environmental variables at fine spatial scales influence the distribution and abundance of ferns (Karst *et al.*, 2005), paired sites were located within 5–10 m of plots where *B. pumicola* occurred. Paired sites were chosen in areas where no *B. pumicola* had been observed for the past three consecutive years. At each site, 10 1-m² plots (5 with *B. pumicola*) were chosen at random and sampled for environmental variables (Elzinga *et al.*, 1998). At LE and LW with larger populations, 10 plots with *B. pumicola* and 10 without were sampled.

Populations were mapped using a Garmin GPSmap 60CSx (Garmin International, Olathe, Kansas). Maps were created in ArcGIS v. 9.3 (ESRI, Redlands, California). Elevation was determined with the GPS unit and field checked with USGS 7-minute topographic maps (Crater Lake East, Crater Lake West, Broken Top, Mt. Bachelor, East Lake, and Mt. Shasta topographic

quadrangles). Slope was determined with a Suunto clinometer (Suunto Inc., Vantaa, Finland) and aspect with a Silva Ranger compass (Johnson Outdoors Inc., Racine, Wisconsin). In each 1-m² plot, all plant species were identified and stems counted.

Data collection began 22 Jun 2009 and ended 28 Aug 2009 when most *B. pumicola* plants had senesced. To minimize researcher impact on fragile pumice substrates, workers wore light-weight, soft-soled "pumice shoes" and stooped beside plots during data collection. Sampling was carried out in relation to *B. pumicola* phenology, beginning at the lowest elevation site and moving up in elevation so that all populations were sampled during the spore-release stage (Johnson-Groh and Lee, 2002). Sampling was conducted once during the growing season which has been found to be sufficient in subalpine sites to detect most *Botrychium* plants (Lesica and Ahlenslager, 1996).

Botrychium pumicola spore dispersal.—Spores were isolated from soil samples by centrifugation (Mason and Farrar, 1989; Brundrett *et al.*, 1996; Fig. 2D). Johnson-Groh *et al.* (2002) sampled soils for *Botrychium* gametophytes to a depth of 10 cm, and because density of fern spores was higher in the upper 10 cm of soil (del Ramirez-Trejo *et al.*, 2004), soil samples for spore isolation were taken to a depth of 10 cm, and stored at 15–21°C for 90–120 days until processing. For each sample, 250 mL of soil was mixed with 250 mL distilled water and soaked for 30 min. Wetted soil was decanted through Tyler Standard Screen soil sieves (W.S. Tyler, Mentor, Ohio) and flushed with tap water. Number 200 (75 µm opening) and 325 (43 µm opening) sieves plus the catch basin, were used to capture *B. pumicola* spores (70 µm tetrad). Sievings were placed in 50-ml centrifuge tubes and spun in a Sorvall RC 5C Plus Centrifuge (Thermo Fisher Scientific Inc., Waltham, Massachusetts) for 5 min at 2000 RPM. Supernatant was discarded, and the pellet resuspended in 50% sucrose and centrifuged (3 min, 2000 RPM). Floating material was transferred to microscope slides and examined using a Leica DMLB compound microscope. Spores were photographed using a SPOT Insight digital camera (Diagnostic Instruments, Inc., Sterling Heights, Michigan). Although *Botrychium lanceolatum* (Gmelin) Angström subsp. *lanceolatum* was present at LE, and *B. multifidum* Gmelin and *B. simplex* Hitchcock occurred 4 km from DO, these species release spores individually, while *B. pumicola* spores usually remain in tetrads (Wagner, 1998; Farrar, 2011). To ensure accurate species identification, only spores in tetrads were counted to determine spore density of *B. pumicola*. Spore counts were binned as 0, 1 (1–5), 2 (6–20), 3 (>20).

Airborne spores were trapped during peak spore release (10 August through 8 September) at SK, DU, and north of LE (LNE), a site with 300 *B. pumicola*. Spore traps were constructed by placing 5 cm of double-sided cellophane tape on glass microscope slides and either laying them on the ground or attaching slides vertically to a metal wire (Peck *et al.*, 1990). At DU, spore traps were installed in a symmetrical array from an isolated *B. pumicola*: two horizontal spore traps at 0.5 cm, four vertical traps at 10, 20, 30 cm, and 1, 2, and 3 m. In addition four vertical traps were placed on a north-south axis 5 and 10 m from the eastern edge of the population in line with prevailing winds. At SK, spore

traps were installed in a symmetrical array from an isolated clump of two *B. pumicola* individuals: four horizontal traps at 0.5 cm, four vertical traps radiating out from two *B. pumicola* plants at 10, 20, and 30 cm and four vertical traps at 1, 2, and 3 m. In addition five vertical traps were placed on a north-south axis 5, 10 and 20 m from the southeast edge of the population and four vertical traps 5 m from the northern edge of the population in line with prevailing winds. At LNE, five vertical spore traps were installed 1, 5, 10 and 20 m from southern edge of the population and 1, 5, and 10 m from western edge of the population in line with prevailing winds. Spore traps were recovered after two weeks, examined dry with light microscopy, and spore tetrads counted.

Soil analyses.—Soil temperature readings were taken in each 1-m² plot 10 cm below the soil surface using a Taylor Commercial digital probe thermometer model 9842 (Taylor Precision Products, Las Cruces, New Mexico; accuracy range $\pm 0.6^\circ$ C). To avoid damaging *B. pumicola* plants, temperature readings were taken 2–3 cm from a *B. pumicola* plant in plots with *B. pumicola* and from another plant species in plots without *B. pumicola*.

Soil samples (500-mL) were taken at depths of 10–12 cm from one randomly selected plot with *B. pumicola* and one plot without *B. pumicola* for each population except for SH which was not sampled. The sample at DU was taken 10 cm from the *B. pumicola* individual, to avoid removing any potential germlings from this small population. Samples were stored at 0° C and transported on ice to the Soil Analytical Laboratory at Oregon State University, Corvallis, USA, for testing of soil moisture, organic matter, pH, P, K, Mg, Na, Ca, NO₃, NH₄, soil density and particle size using the methods of Horneck *et al.* (1989).

Data analyses.—Environmental variables were divided into abiotic factors including soil pH, soil nutrients, soil textural properties and site characteristics (slope, aspect and elevation), and biotic factors including *B. pumicola* phenology, other plant species, and nearest neighbors (*B. pumicola* and closest associated plant species). The 95% confidence interval was used as the level of significance for all analyses. Data were analyzed with a Wilcoxon matched pairs test using Statistica v.7 software (StatSoft Inc., Tulsa, Oklahoma) to determine if plots with and without *B. pumicola* differed.

A multivariate analysis of environmental variables was conducted using PC-ORD v. 5 software (MJM Software, Gleneden Beach, Oregon). Using Non-Metric Multidimensional scaling (NMS), ordinations were run for each group of environmental variables to demonstrate any separation between plots with and without *B. pumicola*. All ordinations used the Sørensen (Bray-Curtis) distance measure. McCune and Mefford (2006) recommend removing outliers due to their potential to skew data, so an outlier analysis was conducted for all environmental variables, outliers with high calcium were removed, and the NMS test was re-run. Differences were analyzed using Multi-Response Permutation Procedures (MRPP) in PC-ORD to test the hypothesis of no difference between two or more groups of environmental variables. As with NMS, outliers with high calcium were removed, and the test rerun.

TABLE 2. Soil temperature (mean) of plots with and without *Botrychium pumicola* and test for differences by Wilcoxon Matched Pairs test for alpine and subalpine populations in the Cascade Range of central and southern Oregon.

| Population | Mean soil temp °C | |
|---------------------|----------------------------|---------------------------|
| | <i>B. pumicola</i> present | <i>B. pumicola</i> absent |
| Llao West | 17.87 | 20.81 |
| Llao East | 10.25 | 14.35 |
| Broken Top | 20.58 | 20.32 |
| Dome | 22.32 | 23.92 |
| Grotto Cove | 24.30 | 20.68 |
| Dutton Ridge | 15.60 | 15.70 |
| Skell Head | 19.10 | 18.68 |
| Mt. Bachelor | 13.74 | 15.08 |
| Mean (sd) | 17.97 (4.48) | 18.69 (3.37) |
| <i>P</i> (Wilcoxon) | 0.006 ^a | |

^a significant at $P < .05$

Associated plant species data were modified by removing species occurring in fewer than 5% of plots. In order to minimize the effect of disproportionately large numbers in the plant associate data set, a relativization was applied. Species that occurred in only one population (singletons) were removed from the data set. Species-area curves were constructed in PC-ORD 5.0 using Sørensen distance measure (McCune and Grace, 2002).

Nearest neighbor analysis was conducted to determine the spatial dispersion between each *B. pumicola* individual and the nearest conspecific or other plant species. A Nearest Neighbor Index (NNI) was calculated (Clark and Evans, 1955). Values approaching zero indicate a clumped distribution, values near one indicate a random distribution, and a value near 2.15 indicates a uniform distribution.

Aspect measurements for each population were binned into one of eight cardinal directions: North (N) 336–25°; North East (NE) 26–70°; East (E) 71–110°; South East (SE) 111–160°; South (S) 161–200°; South West (SW) 201–250°; West (W) 251–290°; North West (NW) 291–335°. Binned aspects were compared with a Chi-square Goodness of Fit Test (Minitab v.15, Minitab, State College, Pennsylvania).

RESULTS

Study sites ranged in elevation from 2100 to 2760 m. Aspect ranged from 12° to 290° on slopes from relatively flat to 27° (Table 1). *Botrychium pumicola* occurred on binned aspects: west (five populations); south and southwest (four populations each); southeast (two populations); north, northeast and east (one population each). No populations occurred on binned northwest aspects (Chi-square Goodness of Fit, $P = 0.045$).

Abiotic factors.—Soil temperatures were lower in plots with *B. pumicola* (Wilcoxon Matched Pairs, $P = 0.006$; Table 2). Soil textural properties of

TABLE 3. Soil textural properties in paired plots with *B. pumicola* present (P) or absent (A) compared by Wilcoxon Matched Pairs test for alpine and subalpine populations in the Cascade Range of central and southern Oregon.

| Population | Density (g/L) | | OM % | | H ₂ O % | | Gravel % | | Sand % | | Silt % | | Clay % | |
|------------------------------|-------------------|-------|------|-----|--------------------|------|----------|------|--------|------|--------|------|--------|-----|
| <i>B.</i> <i>pumicola</i> | P | A | P | A | P | A | P | A | P | A | P | A | P | A |
| LW | 371.3 | 505.1 | 2.8 | 1.5 | 4.3 | 1.0 | 23.7 | 17.9 | 91.3 | 93.8 | 7.5 | 3.8 | 1.3 | 2.5 |
| LE | 371.7 | 559.6 | 2.5 | 0.9 | 11.9 | 8.1 | 20.0 | 35.4 | 91.3 | 97.5 | 5.0 | 1.3 | 3.8 | 1.3 |
| BR | 405.6 | 508.4 | 2.8 | 2.7 | 2.5 | 1.9 | 34.7 | 49.1 | 83.8 | 82.5 | 12.5 | 13.8 | 3.8 | 3.8 |
| DO | 342.5 | 404.5 | 2.3 | 2.8 | 5.5 | 9.2 | 56.0 | 67.0 | 85.0 | 81.3 | 12.5 | 15.0 | 2.5 | 3.8 |
| GR | 532.4 | 460.4 | 1.0 | 0.9 | 1.0 | 1.1 | 25.1 | 25.3 | 92.5 | 97.5 | 3.8 | 1.3 | 3.8 | 1.3 |
| DU | 499.7 | 542.1 | 1.8 | 1.5 | 4.3 | 7.5 | 13.7 | 31.3 | 92.5 | 92.5 | 5.0 | 6.3 | 2.5 | 1.3 |
| CS | 465.7 | 558.8 | 1.8 | 1.7 | 1.9 | 1.4 | 36.9 | 37.4 | 90.0 | 92.5 | 7.5 | 3.8 | 2.5 | 3.8 |
| SK | 357.0 | 483.6 | 1.0 | 1.0 | 5.1 | 9.4 | 21.7 | 23.0 | 96.3 | 90.0 | 2.5 | 6.3 | 1.3 | 3.8 |
| BA | 494.7 | 493.7 | 1.9 | 1.9 | 9.0 | 11.3 | 20.4 | 20.3 | 86.3 | 82.5 | 10.0 | 10.0 | 3.8 | 3.8 |
| Mean | 426.7 | 501.8 | 2.0 | 1.7 | 5.1 | 5.6 | 28.0 | 34.1 | 89.9 | 90.0 | 7.4 | 6.8 | 2.8 | 2.8 |
| sd | 71.7 | 49.8 | 0.7 | 0.7 | 3.5 | 4.2 | 12.8 | 15.7 | 4.1 | 6.4 | 3.7 | 5.1 | 1.0 | 1.2 |
| <i>P</i> | 0.03 ^a | | 0.07 | | 0.68 | | 0.05 | | 0.89 | | 0.48 | | 0.80 | |

^a Significant at $P < .05$

percent sand, silt, or clay did not differ between paired plots with and without *B. pumicola*; soil density was lower in plots with *B. pumicola* in seven out of nine pairs (Wilcoxon Matched Pairs, $P = 0.032$; Table 3). Paired plots did not differ in pH, phosphate, magnesium, sodium, calcium, nitrate, ammonium (Table 4), or in percent organic matter (Table 3). Potassium was higher in plots with *B. pumicola* (Wilcoxon Matched Pairs, $P = 0.024$; Table 4), although similar values of K were found in some plots without *B. pumicola*.

NMS ordination of all abiotic factors showed a high degree of overlap between plots with and without *B. pumicola*. No differences in abiotic factors were observed using Multi-Response Permutation Procedure (MRPP) for all populations ($P = 0.180$). When outliers (values >2 standard deviations from the mean) were removed, differences in abiotic factors were significant (MRPP, $P = 0.049$; Fig. 3).

Biotic factors.—Thirty-five plant species occurred in plots with *B. pumicola* and 32 in plots without *B. pumicola* (Table 5). The most abundant species was *Raillardella argentea*, and the most frequent were *Carex breweri* and *Lupinus lepidus*. No plant species, except *B. pumicola*, was present at all sites. Species-area curves indicated that plots with *B. pumicola* were similar in species richness of associated plants (first order jackknife estimate, 44.9) to plots without *B. pumicola* (first order jackknife estimate, 42.5). For an accumulated eight sites, species-area curves of plots with and without *B. pumicola* had 32 associated species. The density of associated plants showed a high range of variability in plots with and without *B. pumicola* (Table 5).

NMS ordination of all plant associates showed differences between plots with and without *B. pumicola* (MRPP, $P = 0.015$). When abundance values were relativized to minimize the effect of plots with disproportionately large

TABLE 4. Soil pH and soil nutrients in paired plots with *B. pumicola* present (P) or absent (A) compared by Wilcoxon Matched Pairs test for alpine and subalpine populations in the Cascade Range of central and southern Oregon.

| Population | pH | | P (mg/kg) | | K (mg/kg) | | Mg (mg/kg) | | Na (mg/kg) | | Ca (mg/kg) | | NO ₃ (mg/kg) | | NH ₄ (mg/kg) | |
|----------------------------|------|-----|--------------|------|-------------------|------|---------------|-------|---------------|------|---------------|-------|----------------------------|-----|----------------------------|-----|
| | P | A | P | A | P | A | P | A | P | A | P | A | P | A | P | A |
| <i>Botrychium pumicola</i> | | | | | | | | | | | | | | | | |
| LW | 6.3 | 6.5 | 30 | 27 | 88 | 63 | 47 | 54 | 21 | 15 | 405 | 217 | 1.7 | 0.8 | 2 | 1.4 |
| LE | 6.5 | 6.5 | 20 | 35 | 88 | 47 | 59 | 31 | 19 | 15 | 513 | 117 | 1.3 | 0.9 | 1.6 | 1.0 |
| BR | 6.3 | 5.7 | 40 | 50 | 82 | 39 | 129 | 23 | 20 | 13 | 461 | 45 | 1.3 | 1.3 | 4.9 | 1.2 |
| DO | 6.3 | 6.3 | 10 | 12 | 119 | 98 | 136 | 145 | 27 | 22 | 597 | 589 | 3.1 | 3.2 | 2.3 | 1.7 |
| GR | 6.2 | 6.5 | 48 | 43 | 52 | 47 | 27 | 26 | 29 | 19 | 95 | 112 | 0.3 | 0.8 | 0.9 | 1.0 |
| DU | 6.3 | 6.4 | 45 | 39 | 71 | 76 | 31 | 38 | 13 | 18 | 167 | 171 | 0.09 | 1.3 | 1.2 | 1.4 |
| CS | 6.6 | 6.5 | 12 | 14 | 91 | 90 | 111 | 122 | 14 | 19 | 329 | 372 | 0.7 | 1.0 | 1.1 | 1.2 |
| SK | 6.5 | 6.4 | 30 | 31 | 63 | 59 | 43 | 38 | 28 | 19 | 176 | 157 | 0.4 | 0.7 | 1.0 | 1.0 |
| BA | 6.5 | 6.4 | 19 | 15 | 107 | 82 | 194 | 488 | 24 | 20 | 1230 | 2010 | 0.6 | 0.9 | 1.5 | 1.4 |
| Mean | 6.4 | 6.4 | 28.2 | 29.6 | 84.6 | 66.8 | 86.3 | 107.2 | 21.7 | 17.8 | 441.0 | 421.0 | 1.1 | 1.2 | 1.8 | 1.3 |
| sd | 0.1 | 0.3 | 14.0 | 13.6 | 20.8 | 20.8 | 58.4 | 149.4 | 5.8 | 2.9 | 341.0 | 618.0 | 0.9 | 0.8 | 1.2 | 0.2 |
| <i>P</i> | 0.87 | | 0.95 | | 0.02 ^a | | 0.59 | | 0.09 | | 0.59 | | 0.40 | | 0.12 | |

^a Significant at $P < 0.05$

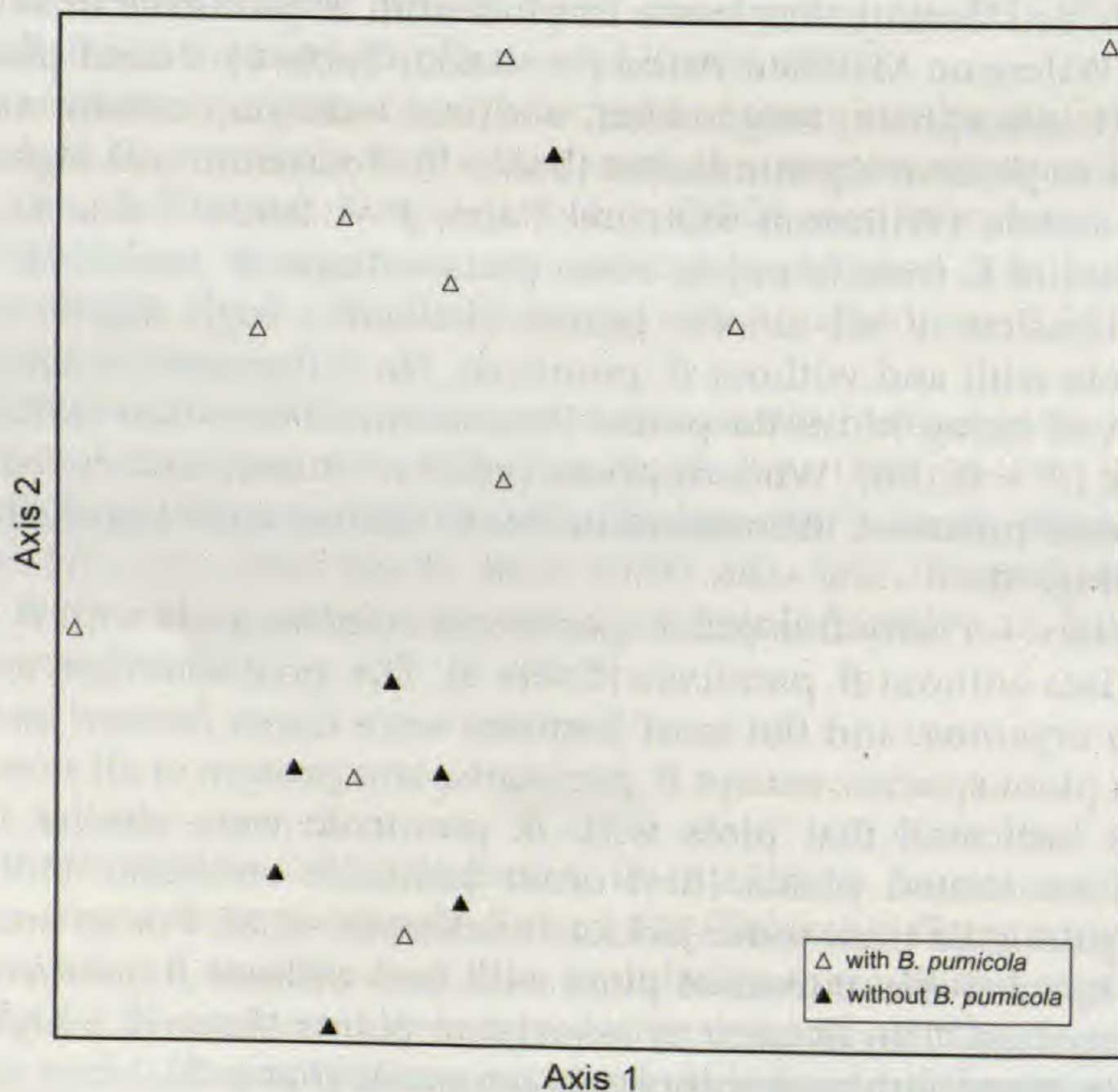


FIG. 3. NMS ordination for *Botrychium pumicola* populations in the Cascade Range of central and southern Oregon. All abiotic factors ordinated by site; outliers with high calcium removed; Δ = with *B. pumicola*, \blacktriangle = without *B. pumicola*.

numbers, plots with and without *B. pumicola* still differed (MRPP, $P = 0.007$). When associated plant species occurring in fewer than 5% of plots were removed, plots with and without *B. pumicola* differed in community composition (MRPP, $P = 0.018$; Fig. 4). NMS ordination of all plant associates per m^2 with singletons removed showed no differences between sites with and without *B. pumicola* (MRPP, $P = 0.875$). NMS ordination of abiotic factors plus plant associates with singletons removed showed no difference between plots with and without *B. pumicola* (MRPP, $P = 0.875$).

Nearest-neighbor distances between *B. pumicola* conspecifics ranged from 9.1 to 71.6 cm with clumped and random dispersions (Table 6). In four populations, NNIs were below 1.00 indicating clumping; in five populations, NNIs of 1.10–1.33 indicated a random distribution. Nearest neighbor distances between *B. pumicola* individuals and the nearest associated plant species ranged from 1.6 to 15.4 cm (Table 6). Nearest neighbor indices between *B. pumicola* individuals and the nearest other plant species showed a clumped dispersion ($NNI < 1.00$) in all populations except at BR which had an NNI of 2.04 indicating a uniform dispersion (Table 6).

Spore tetrads of *Botrychium pumicola* were found in soil samples from plots with *B. pumicola* (Fig. 2D) at LE (20); LW (1); SK (4); CN (1); CS (2); BR and BA (1 each) and in plots without *B. pumicola* at GR (3); BA (1). No *B. pumicola* spores were found at DU. One single spore (not in tetrad) was found during sampling of BR (*B. pumicola* present) soils. Spore tetrads were found on spore traps at distances up to 10 m. At SK, spores lodged on slides 10 m from the source plant with most spores lodging within 30 cm. At DU spores lodged on slides within 1 m from the source plant. At LNE spores lodged on slides 10 m from the source plant with most spores lodging within 5 m (Fig. 5).

DISCUSSION

Microsite factors.—In paired plots with and without *B. pumicola*, K levels were higher in the plots with *B. pumicola*. Potassium is essential for plant growth, development and regulation of stomata, with the role of K in photosynthesis becoming more important at higher CO_2 levels and light intensity (Larcher, 2002; Cakmak, 2005). A higher K level may help *B. pumicola* regulate water loss in habitats where plants are subject to low summer precipitation, intense UV radiation, temperature extremes, and desiccating winds. Potassium is essential for spore germination in ferns (Miller and Wagner, 1987). Lower K levels may decrease gametophyte success in habitats that appear suitable for *B. pumicola*.

Although several *Botrychium* species are associated with calcareous habitats (Cayouette and Farrar, 2009; Zika *et al.*, 2002), *B. pumicola* is not a calcicole, and calcium did not differ between sites with and without *B. pumicola*.

Soil density was lower in plots with *B. pumicola*. Greater porosity may enable *B. pumicola* spores to move down the soil column. This may assist spores in finding the darkness necessary for germination and the AM fungal partners for continued growth (Johnson-Groh, 2002; Whittier, 2006).

TABLE 5. Plant density (stems per m²) at alpine and subalpine sites with *Botrychium pumicola* present (P) and absent (A) in the Cascade Range of central and southern Oregon and northern California.

| <i>Botrychium pumicola</i> (P/A) | P | A | P | A | P | A | P | A | P | A | P | A | P | A | P | A | P | A | P |
|--|------|------|------|------|------|-----|------|------|-----|----|----|------|------|------|------|------|------|----|-----|
| Plant Associate | LW | LW | LE | LE | BR | BR | DO | DO | GR | GR | DU | DU | CN | SK | SK | BA | BA | SH | |
| <i>Achillea millefolium</i> | | | | | 34.4 | 9 | 11.2 | | | | | | | | | | | | |
| <i>Achnatherum occidentale</i> | | | | | | | | 5.2 | 6.6 | 20 | 20 | 0.2 | 0.2 | 8 | 0.8 | | | | |
| <i>Agoseris</i> sp. | 20.1 | 8.8 | 6.5 | 4.7 | | 1.2 | 2.2 | | 2.4 | | | 0.4 | | 0.6 | | | | | |
| <i>Antennaria alpina</i> var. <i>media</i> | 7.3 | | 4.4 | 0.3 | | | | | | | | | | 12.8 | 13.8 | | | | |
| <i>Antennaria geyeri</i> | | | | | | | | 3.6 | | | | | | | | | | | |
| <i>Antennaria rosea</i> | | 10.6 | | | | | | | 2.4 | | | 15.8 | | | | | | | |
| <i>Arabis divaricarpa</i> | | | | | | | | | | | | | | | | 0.2 | | | |
| <i>Arabis lyallii</i> | | | 0.2 | | | | | | 0.2 | | | | | | | | | | |
| <i>Arabis platysperma</i> | | | | | | | | | 0.4 | | | | | | | | | | |
| <i>Boechera horizontalis</i> | 0.2 | 0.1 | | | | | | | | | | 0.2 | | 5.2 | 1.4 | 0.4 | | | |
| <i>Calyptridium umbellatum</i> | | | | | | | | 1 | 1 | | | | | 3.6 | 5.2 | 1.4 | 0.4 | | |
| <i>Castilleja applegatei</i> | | | | | | 12 | 6.8 | | | | | | | | | | | | |
| <i>Castilleja arachnoidea</i> | 8.5 | 3 | 4.3 | 0.6 | 4.4 | | | | | | | | | | | | | | |
| <i>Carex breweri</i> | 24 | 20.7 | 40.2 | 35.7 | 24.4 | | | 14.4 | 6.4 | 10 | 12 | 10.4 | | 6.8 | 9.2 | 15.2 | 29.2 | | |
| <i>Carex inops</i> | 12.4 | 2.1 | | | | | | | | | | | | | | | | | |
| <i>Collinsia parviflora</i> | | | | | | 3.6 | 0.2 | | | | | | | | | | | | |
| <i>Elymus elymoides</i> | 2.5 | 8.9 | 7 | 14.4 | 1.4 | | | 3.2 | 3.2 | 0 | 0 | 7.6 | | 4.8 | 6.2 | | | | |
| <i>Ericameria nauseosa</i> | | | | | | | | 0.6 | | | | | | | | | | | |
| <i>Erigeron compositus</i> | | | | | | | | 1.4 | | | | | 1 | 0.2 | 0.8 | 1 | | | |
| <i>Erigeron elegantulus</i> | | | | | | | | | | | | | | | | | | | |
| <i>Eriogonum ovalifolium</i> | 0.1 | 0.9 | 1.1 | 5.4 | | | | 0.2 | 0.6 | | | | 24.4 | 17.4 | 25 | | | | |
| <i>Eriogonum pyrrolifolium</i> | | | | | 0.6 | | | | | | | | 3.6 | 1.2 | | | | | |
| <i>Eriogonum umbellatum</i> | 4.8 | 7.7 | 5 | 8.9 | 4 | 2.4 | 0.6 | 1 | 0.8 | | | | 2 | 3.2 | 2 | | | | |
| <i>Eriophyllum lanatum</i> | | | | | | | | | 0.2 | | | | | 0.6 | 1.2 | | | | |
| <i>Festuca idahoensis</i> | | | | | | | | | | | | | 2.2 | | | | | | |
| <i>Gayophytum diffusum</i> | | | | | | | | | | | | | | | | | | | |
| <i>Holodiscus microphyllus</i> | | | | | | 3.8 | 0.8 | | | | | | | | | | | | 1.5 |
| <i>Hulsea nana</i> | | | | | | | | | | | | | | | | | | | |
| <i>Ipomopsis congesta</i> | | | | | | | | 1.6 | 0.8 | | | | | 2.4 | 3.2 | 0.2 | 0.4 | | |

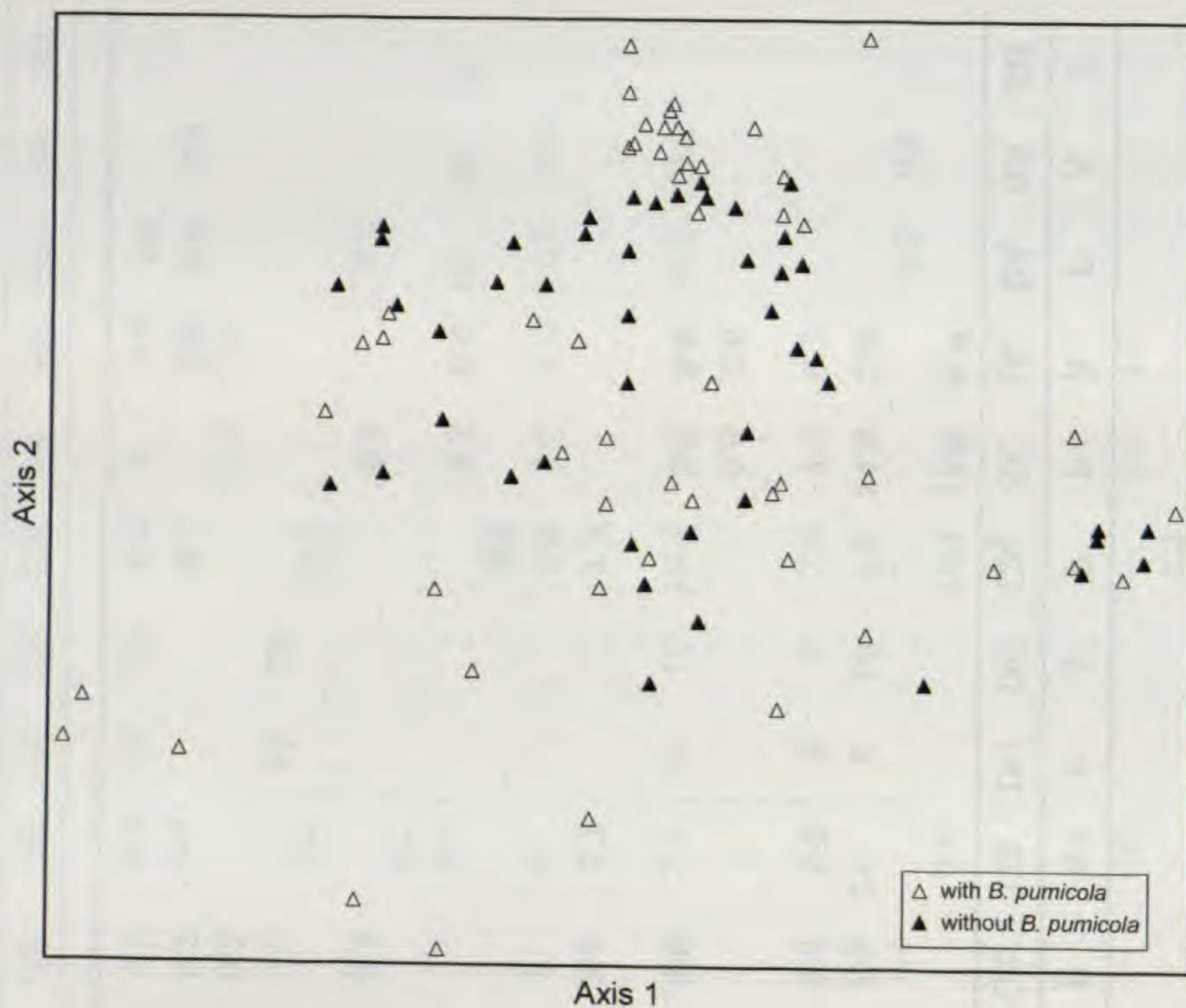


FIG. 4. NMS ordination for *Botrychium pumicola* populations in the Cascade Range of central and southern Oregon. All plant associates in greater than 5% of plots, five replicates per site; ten replicates for Llao Rock; Δ = with *B. pumicola*, \blacktriangle = without *B. pumicola*.

TABLE 6. Nearest neighbor distances and indices for alpine and subalpine populations of *Botrychium pumicola* in the Cascade Range of central and southern Oregon and northern California. Average nearest neighbor distance (mean and sd) and index (NNI) between adjacent plants of *B. pumicola* and between *B. pumicola* and the nearest other species. Dutton Ridge (DU) dispersion could not be calculated due to a sample size of one. Values near zero indicate clumped distribution, values near one indicate random distribution, and values near 2.15 indicate uniform distribution.

| Population | Nearest neighbor distance (cm), mean (sd) | | Nearest neighbor index | |
|------------|--|--|--|--|
| | Between adjacent <i>B. pumicola</i> plants | Between <i>B. pumicola</i> and other species | Between adjacent <i>B. pumicola</i> plants | Between <i>B. pumicola</i> and other species |
| DU | n/a | 5.0 | n/a | 0.74 |
| SH | 9.1 (5.8) | 1.6 (0.1) | 0.34 | 0.10 |
| BR | 12.7 (7.5) | 15.4 (8.2) | 0.61 | 2.04 |
| DO | 13.3 (4.7) | 5.5 (2.7) | 0.68 | 0.95 |
| BA | 16.4 (6.5) | 6.7 (5.7) | 0.74 | 0.73 |
| LW | 17.5 (4.9) | 2.5 (1.2) | 1.10 | 0.69 |
| GR | 71.6 (37.3) | 5.0 (2.6) | 1.15 | 0.68 |
| CN | 22.9 (2.3) | 4.4 (1.3) | 1.17 | 0.88 |
| LE | 25.1 (13.8) | 2.3 (0.8) | 1.22 | 0.66 |
| SK | 44.6 (38.5) | 4.6 (1.8) | 1.33 | 0.86 |

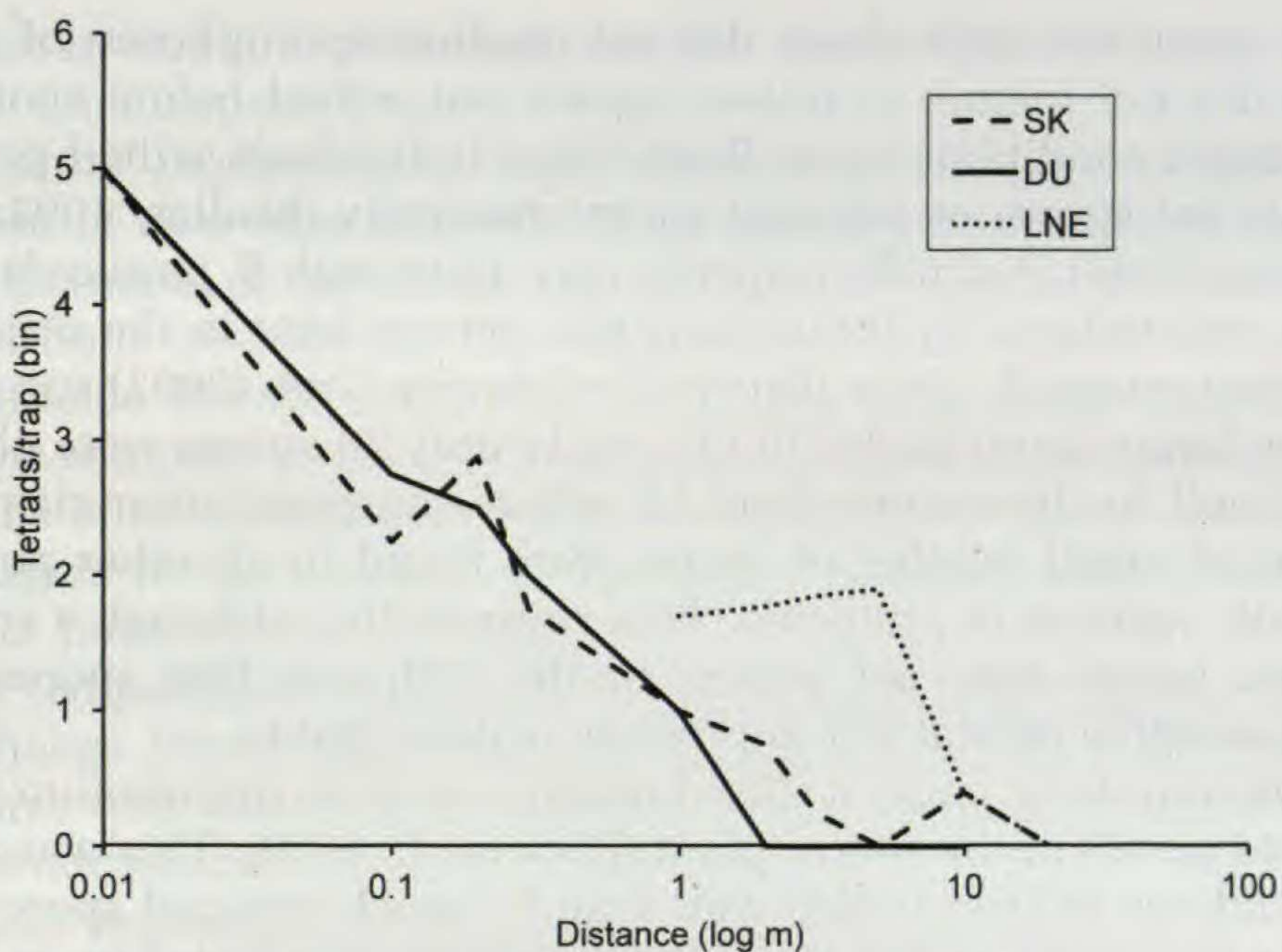


FIG. 5. Dispersal distances of *Botrychium pumicola* spore tetrads in Crater Lake National Park, Oregon. Dutton Ridge (DU); Skell Head (SK); Llao Rock Northeast (LNE).

Microscale slope and aspect may influence soil moisture and soil temperature creating patches of suitable habitat. *Botrychium pumicola* populations occurred on slopes of 0–27°. Populations of *B. pumicola* occurred on all aspects except northwest, with south, southwest, and west aspects more common. Snow distribution, snow pack duration and relief-driven summer precipitation strongly influence moisture availability during the growing season (Körner, 1999). Soil temperatures were lower in plots with *B. pumicola*.

Botrychium pumicola grows with diverse herbaceous perennial plants typical of south-central Cascadian high-elevation pumice fields and volcanic rubble, commonly *Carex breweri*, *Elymus elymoides*, *Eriogonum umbellatum* and *Lupinus lepidus* (Roe-Andersen, 2010). No plant species consistently occurred with *B. pumicola*. The difference in plant community composition in plots with and without *B. pumicola* was driven by singletons, which when removed showed no difference between plots. The high density of *Raillardella argentea* at Llao Rock with the largest *B. pumicola* population ($n > 1300$) suggests that it may be an important mycorrhizal network partner.

Nearest neighbor analysis of *B. pumicola* and its nearest plant associate showed that in all populations, except at Broken Top, the plants had clumped dispersion. A nearest neighbor analysis of *B. pumicola* with conspecifics showed clumped and random dispersions. Conspecific clumped distribution suggests that gemmae and spores falling close to the parent plant dominate reproduction and that longer distance dispersal and establishment are less important. Clumping could also facilitate mycorrhizal inoculation of spores (Johnson-Groh and Lee, 2002). Random distribution indicates that *B. pumicola* is not required to be near a conspecific (or other AM host) to establish successfully.

Some *B. pumicola* individuals did not produce sporophores; of those that did, some did not mature to release spores but wilted before spore release. During drought conditions some *Botrychium* individuals wilted prematurely and did not set spores or released spores passively (Muller, 1992; Johnson-Groh and Lee, 2002). A similar response may occur with *B. pumicola* in the dry conditions encountered by individuals that emerge later in the season.

Botrychium pumicola spore dispersal.—Johnson-Groh (2002) suggested that *Botrychium* forms spore banks. In this study only 20 spores were observed in 250 mL of soil in the sample from LE where the population size is >1000 individuals. A small number of spores were found in all other populations, even in plots without *B. pumicola*. This suggests that although a small spore bank forms, spores may not persist in the soil, and that spores reaching apparently suitable habitat fail to produce mature plants.

In a spore trap study under a closed canopy, most *B. virginianum* spores fell within three meters of the source plant (Peck *et al.*, 1990). This was consistent with the findings of Dyer (1994) who found a much reduced spore bank two meters from the source. In this study, spore tetrads were found up to 10 m from a source plant, with most tetrads lodged within 30–100 cm. This supports a leptokurtic pattern of *Botrychium* spore dispersal with most spores lodging close to source plants (Peck *et al.*, 1990).

Rarity.—According to Rabinowitz's (1981) classification of species abundance, *B. pumicola* falls into the category of a species with both a narrow geographic range and narrow habitat specificity. Rarity of a species may be influenced by the size and number of habitable sites, by the carrying capacity of the sites, and by the length of habitability and the probability of dispersal to those habitats (Harper, 1981). *Botrychium pumicola* appears limited to volcanic substrates with appropriate soil nutrients, temperature, and density, and may also be limited by spore dispersal into suitable habitat. Due to the windy nature of exposed volcanic substrates, spores may periodically land in suitable habitat making the potential for colonizing distant habitats seem high. However, the fragmented distribution of populations suggests that, although dispersal occurs, the species encounters limitations in its ability to establish. High mortality from gametophyte to emergent sporophyte has been observed in other *Botrychium* species (Johnson-Groh *et al.*, 2002). If *B. pumicola* spores reach suitable habitat, the potential for failure of spores, gametophytes and juvenile sporophytes may be high.

Botrychium pumicola may be a "neo-endemic," a relatively new species that has not had time to expand its range (Fiedler, 2001). It may also be recovering from bottleneck effects due to past volcanic eruptions. The rediscovery of *B. pumicola* on Shastina Cone (Farrar, 2011; Farrar, 2012) adds a fascinating layer to the ecology of *B. pumicola* and opens a new chapter in the evolution and distribution of the species thought to be an Oregon endemic. Although *B. pumicola* is restricted to select volcanic habitats in the Cascade Range of central and southern Oregon and extreme northern California, the Shastina Cone population confirms that the species is not restricted to pumice substrates as previously reported (Wagner, 1990; Wagner and Wagner, 1993). Because the Shastina Cone plants have the same single genotype as the Oregon

plants (Farrar, 2011), a rare long distance spore dispersal event may have supported colonization of newer volcanic substrates in southern Oregon.

Conservation implications.—Long distance spore dispersal may allow for colonization of new sites, however, *B. pumicola* spore dispersal is primarily local. Furthermore, *B. pumicola* may have specific microsite habitat requirements that limit colonization. The fragmented distribution of the species and limited suitable habitat may preclude *B. pumicola* from establishing new populations at a pace to match the rate of floristic shifts accompanying climate change. The effects of climate change on *Botrychium* species are a concern of land managers in the western United States (Anderson and Cariveau, 2004). *Botrychium pumicola* may be negatively affected by increased CO₂, increased maximum temperatures and decreased snow water equivalent expected with climate change (Mote, 2003; Mote *et al.*, 2005). Predicted higher maximum temperatures with concomitant decrease in summer moisture levels may push the species northward in latitude and up in elevation. Alternatively, a downward shift in plant species distribution may occur with changes in water availability (Crimmins *et al.*, 2011). Consequences of either scenario are a reduction in suitable habitat islands for colonization and increased risk of local extinction.

Conclusions.—Our results suggest that microsite factors (soil nutrients, soil density, nearest neighbor dispersion) and dispersal limitations are likely key factors influencing the distribution of *Botrychium pumicola*. The fragmented distribution of *B. pumicola* populations results from a convergence of abiotic and biotic factors that create habitat islands appropriate for the growth and development of *B. pumicola* gametophytes, sporophytes and their AM fungal symbionts. Narrow spore germination requirements combined with dispersal limitations may influence population expansion of obligate mycorrhizal ferns such as *B. pumicola*.

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